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WHO Respiratory Disease Survey in Children

A Serological Study

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This paper is a report on the first (serological) phase of a study organized by WHO in collaboration with the WHO International Reference Centre for Respiratory Virus Diseases other than Influenza in Bethesda, Md., USA, to define the viral etiology of severe respiratory infections in children, particularly in tropical areas. Paired sera from 528 children up to 5 years old admitted to hospital with severe respiratory illness of probable viral etiology were collected in 10 countries and sent frozen to the International Reference Centre, where standard complement-fixation tests were made for the following agents: parainfluenza virus types 1, 2 and 3, influenza virus types A and B, adenoviruses, respiratory syncytial virus, Mycoplasma pneumoniae, Coxiella burneti *and psittacosis-ornithosis.*

Some 41% of paired sera showed rising antibody titres for one or more of these agents, multiple infections being observed in 8%. In most of the countries the pattern of infection was similar. RS virus was the most important respiratory tract pathogen of early life, particularly in the first year of life and in cases of bronchiolitis and pneumonia; the parainfluenza viruses were next in importance, particularly in cases of croup, but, in contradistinction to RS virus infections, they were commoner in older children. Influenza, adenoviruses, and M. pneumoniae *were of moderate importance, and* C. burneti *and the psittacosis-ornithosis agents were relatively rare. This pattern is similar to that which has been observed in temperate climates.*

A number of studies have been performed in different countries to assess the etiological role of viruses in respiratory tract illness (Berglund et al., 1965; Chanock & Parrott, 1965; Bukrinskaya & Blumental, 1962; Grayston et al., 1965; Hilleman et al., 1962; Lewis, Lehman & Ferris, 1961; Lewis et al., 1961; McLean et al., 1963; Medical Research Council Working Party on Acute Respiratory Virus Infections, 1965). These studies were carried out mainly in temperate climates. From them a consistent pattern emerged and it has been shown that certain myxoviruses, paramyxoviruses, adenoviruses, rickettsiae, psittacosis-ornithosis agents, and mycoplasmae can provoke respiratory illness. Furthermore it has been found that these illnesses are particularly severe in infancy and childhood and not uncommonly lead to death. Respiratory syncytial (RS) and parainfluenza viruses have been found to be the most important agents, followed by adenoviruses, influenza viruses and *Mycoplasma pneumoniae* (Beem et al., 1962; Berglund

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et al., 1965; Chanock, 1965; Chanock et al., 1962; McClelland et al., 1961; Parrott et al., 1962; Van der Veen, 1963). Rhinoviruses have also been found to be important pathogens although they normally cause mild disease (Medical Research Council Working Party on Acute Respiratory Virus Infections, 1965). In certain areas, *Coxiella burneti* and psittacosis-ornithosis agents have shown to be important respiratory tract pathogens (Medical Research Council Working Party on Acute Respiratory Virus Infections, 1965).

In developing countries, particularly tropical and semitropical ones, information on such infections is rare owing to the lack of laboratory facilities and epidemiological data. The World Health Organization therefore organized a study in close collaboration with the WHO International Centre for Respiratory Virus Diseases other than Influenza in Bethesda, Md., USA, to define the viral etiology of severe respiratory illness in children in several countries in tropidal and semitropical areas.

This study was divided into two phases. In the first serological methods were used, while in the second attempts to recover viruses were also included. The first phase has been in progress for two years and the final results are published here. It is hoped that the seond will be completed in about a year's time.

Laboratories in the following cities participated: Beirut (Lebanon), Bombay (India), Cairo (UAR), Dakar (Senegal), Hong Kong, Kingston (Jamaica), Lima (Peru), New Delhi (India), Porto (Portugal), Port of Spain (Trinidad), and Singapore.

MATERIAL AND METHODS

Composition of study populations

Paired sera from children up to 5 years of age admitted to hospital with acute severe respiratory illness of probable viral etiology were collected, the first specimen as soon as possible after admission to the hospital and the second 10–20 days afterwards.

Altogether pairs of sera from 528 children were studied; the number of patients from the different areas varied from 7 to 100 (Table 1).

The clinical conditions were classified as follows: pneumonia, bronchiolitis, bronchitis, croup, upper respiratory illness (URI), influenza-like illness and

TABLE 1

STUDY POPULATION IN RESPIRATORY DISEASE SURVEY

Area	Age of patients (months)							Clinical diagnosis							
	⩽5	6-11	12-23	24-35	36-59	⩾60	Unknown	Pneumonia	Bronchiolitis	Bronchitis	Croup	URI [a]	Influenza	Other [b]	Total
Cairo			1	26	61	12		6		81			11	2	100
Singapore	10	21	20	12	13		1	19		27		31			77
Hong Kong	7	19	8	11	11			21	9	1	7	17		1	56
Kingston	10	12	25	18	12			37	22	4	5	6		3	77
Port of Spain	22	17	26	10	9			57	17	2	8				84
New Delhi	3	4	13	3	13			22	1	5	2	6			36
Bombay		3	5	2	2			1	1					10	12
Dakar	2	2	11	5	5		1	17		3	2	2		2	26
Beirut	2	1	1	2	1			2	3					2	7
Lima	5	8	7	10	7			20	2	8	6			1	37
Porto	6	4	3	1	2			8	1	2	1	1		3	16
Total	67	91	120	100	136	12	2	210	56	133	31	63	11	24	528

[a] URI = upper respiratory tract infection.

[b] Includes 3 patients with lower respiratory tract disease, 1 with herpangina, 3 with cough, 1 with fever of undetermined origin, 1 with purulent pneumothorax, 1 with febrile gastroenteritis, and 14 in whom a diagnosis was not made.

" other ". The number of cases in each of these categories is shown in Table 1.

Laboratory techniques

Acute- and convalescent-phase sera were tested simultaneously in the WHO International Reference Centre for Respiratory Virus Diseases other than Influenza, Bethesda, by the complement-fixation technique (Kapikian et al., 1961). Four to 16 units of the following antigens were used: parainfluenza virus types 1, 2 and 3, RS virus, influenza A and B, adenovirus, *M. pneumoniae*, *C. burneti*, and psittacosis-ornithosis.

The parainfluenza virus antigens were prepared in the chick-embryo amniotic sac, influenza virus antigens in the chick-embryo allantoic sac, RS virus and adenovirus (type 2) antigens in Hep-2 tissue cultures and *C. burneti* and psittacosis-ornithosis antigens from ether-extracted chick embryo yolk sac. *M. pneumoniae* antigen consisted of a suspension of organisms grown on a glass surface and washed free of growth-medium constituents. Sixteen units of RS virus and *M. pneumoniae* antigens were used to ensure maximum sensitivity for serodiagnosis since it has been found that lesser amounts of antigen often fail to detect a serological response in infected infants (Kapikian et al., 1961). A test was considered positive only if there was at least a 4-fold increase in antibody between the first and second blood specimens.

RESULTS

The results are given in Tables 2-5.

In Table 2 is shown the distribution of positive findings by area and by etiological agent. Of the 528 paired sera tested, 215 (41%) were positive. Of this group 46 infants (8%) had serological evidence of infection with more than one agent; this explains the apparent discrepancy between the individual and total figures in this table and in Tables 3 and 4. Six of the 46 patients who developed antibody to more than one agent had a multiple response which involved only parainfluenza virus antigens. Since heterotypic parainfluenza virus antibody responses occur commonly following parainfluenza infection it is likely that such double responses do not represent double infection (Chanock et al., 1963). The proportion of patients giving positive results varied from 14% to 52% (22% to

TABLE 2

DISTRIBUTION OF PATIENTS WITH RESPIRATORY DISEASE, BY AREA

Area	No. of patients tested	Number with serological evidence of infection											
		Parainfluenza				RS	Influenza		Adeno-viruses	*M. pneu-moniae*	*C. burneti*	Psitta-cosis-orni-thosis	Total
		Total	Type 1	Type 2	Type 3		A	B					
Cairo	100	28	7	9	21	18	14	7	14	3		1	52 (52%)
Singapore	77	6	2		4	18	5		7	6			37 (48%)
Hong Kong	56	4	2	1	3	9			2	6			18 (32%)
Kingston	77	9	4		7	13			3	3			26 (34%)
Port of Spain	84	12	10	3	4	27	1		2	5	1	1	41 (49%)
New Delhi	36	8	4		5	11				1			16 (44%)
Bombay	12	1			1			1					2 (17%)
Dakar	26	3	1		2	1			4				7 (27%)
Beirut	7					1							1 (14%)
Lima	37	6	1		6	2		1	1				8 (22%)
Porto	16	6	1		6	1		1					7 (44%)
Total	528	83 (16%)	32 (6%)	13 (2%)	59 (11%)	101 (19%)	20 (4%)	10 (2%)	33 (6%)	24 (5%)	1 (0.2%)	2 (0.4%)	215[a] (41%)

[a] 46 infants (8%) had serological evidence of infection with more than one agent.

TABLE 3

PERCENTAGE DISTRIBUTION OF PATIENTS WITH RESPIRATORY DISEASE, BY CLINICAL DIAGNOSIS

Clinical diagnosis	No. of patients tested	Percentage with serological evidence of infection											
		Parainfluenza				RS	Influenza		Adeno-viruses	*M. pneu-moniae*	*C. burneti*	Psitta-cosis-orni-thosis	Total
		Total	Type 1	Type 2	Type 3		A	B					
Pneumonia	210	13	6	0.5	9	21	1	0.5	4	5	0.5	0.5	39
Bronchiolitis	56	11	4	2	7	38		2	2	4			52
Bronchitis	133	25	6	7	18	20	10	5	11	3		0.1	47
Croup	31	23	16	6	13	6	3		3	6			32
Upper respiratory tract infection	63	13	6		11	5	3	2	11	6			32
Influenza-like illness	11					9	9			9			27
Other illneses [a]	24	8			8	12		5	5				29
Total	528	16	6	2	11	19	4	2	6	5	0.2	0.4	41[b]

[a] Includes 3 patients with lower respiratory tract disease, 1 with herpangina, 3 with cough, 1 with fever of undetermined origin, 1 with purulent pneumothorax, 1 with febrile gastroenteritis, and 14 in whom a diagnosis was not made.

[b] 8% of patients had serological evidence of infection with more than one agent.

52% if the two areas with the smallest numbers of patients are omitted).

Infections with RS virus were most frequently identified (19%), followed by the parainfluenza viruses (16%), adenoviruses (6%), *M. pneumoniae* (5%), influenza A (4%), influenza B (2%). Psittacosis (0.4%) and *C. burneti* (0.2%) infections were rare. In the parainfluenza group type 3 was commonest (11%), followed by type 1 (6%) and type 2 (2%).

The general pattern described above was not the same in all places—for example, Cairo, Dakar, Lima and Porto showed higher numbers of parainfluenza

TABLE 4

PERCENTAGE DISTRIBUTION OF PATIENTS WITH RESPIRATORY DISEASE, BY AGE

Age (months)	No. of patients tested	Percentage with serological evidence of infection											
		Parainfluenza				RS	Influenza		Adeno-viruses	*M. pneu-moniae*	*C. burneti*	Psitta-cosis-orni-thosis	Total
		Total	Type 1	Type 2	Type 3		A	B					
⩽5	67	7	3	1	4	37							42
6-11	91	9	3		8	27	1		5	4			43
12-23	120	20	8	2	12	12	1		4	7			35
24-35	100	17	7	2	13	16	6	4	12	4	1	1	44
36-59	136	19	7	4	15	14	7	4	7	5		1	41
⩾60	12	25		8	17	8	8	8		8			42
Unknown	2						50		50				100
Total	528	16	6	2	11	19	4	2	6	5	0.2	0.4	41[a]

[a] 8% of patients had serological evidence of infection with more than one agent.

TABLE 5

DISTRIBUTION OF INFECTION OBSERVED DURING THE 4 QUARTERS OF THE YEAR OVER PERIOD OF COLLABORATIVE STUDY

Location	Number of patients tested				Percentage with infection during indicated quarter																											
					Parainfluenza												Influenza								RS virus				Adenoviruses			
					Type 1				Type 2				Type 3				A				B											
	1st quarter	2nd quarter	3rd quarter	4th quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Countries in northern hemisphere [a]	124	88	118	161	9	14	4	8	3	14	3	3	15	7	12	9	3	4	6	4	2	—	3	3	9	28	28	17	3	11	8	5
Countries in southern hemisphere [b]	5	0	25	6	—	—	1	—	—	—	—	—	1	—	5	—	—	—	—	—	—	—	1	—	—	—	2	—	—	—	1	—

[a] Hong Kong, India, Jamaica, Lebanon, Portugal, Senegal, Singapore, Trinidad, United Arab Republic.
[b] Peru (in one case data unknown).

than of RS virus infection—but the real significance of these and other variations shown in Table 2 is difficult to assess on the basis of the numbers of observations recorded. Except in Cairo and Singapore, influenza viruses were rarely detected. Serological evidence of *M. pneumoniae* was found in 6 localities.

In Table 3 the results are shown in relation to the clinical conditions. The proportion of patients whose tests were positive varied from 27% in the group with influenza-like illness to 52% in those with bronchiolitis. In pneumonia and bronchiolitis, RS and parainfluenza viruses were the commonest agents, followed by *M. pneumoniae* and the adenoviruses. In bronchitis the order was: parainfluenza viruses, RS virus, adenoviruses, and influenza viruses. In croup, the association with parainfluenza viruses was very striking, but cases associated with RS virus, *M. pneumoniae*, influenza A and the adenoviruses were also reported. In the upper respiratory tract illnesses (URI) parainfluenza viruses were commonest, followed by adenoviruses, *M. pneumoniae* and RS virus. In the influenza-like illnesses the same proportions of RS virus, influenza virus A and *Mycoplasma* infections were detected.

In Table 4 the results are related to the age of the patients. RS virus infections were commonest in early infancy, but parainfluenza virus infections increased during the second year of life. In the 0–5-months age-group agents other than RS virus and parainfluenza viruses were not detected. Influenza virus and *Mycoplasma* infections became more frequent as the age of the patients increased. Adenoviruses had a more irregular age distribution. Though there was a clear relationship between age and the etiological agent, the proportion of cases giving positive results varied little between age-groups.

The temporal distribution of infections (Table 5) was similar to that observed in temperate areas except for RS virus, which was a frequent cause of lower respiratory tract disease in tropical areas of the northern hemisphere in the summer months. In previous studies in temperate areas of the northern hemisphere, RS virus disease has been rare during the summer. It is also of interest to note that influenza A virus disease occurred as often in the summer as in the other seasons of the year in the northern hemisphere.

DISCUSSION

Although the number of paired sera examined by locality was small, a general picture of the etiological

role of viruses (and some other agents) in respiratory tract illness was obtained. As all the serological tests were made in one laboratory using a standard technique, the results can be compared with a certain degree of confidence.

In all, 41% of the child patients in the survey developed a serological response to one or more of the antigens included in the serological tests. This proportion is probably an underestimate because the complement-fixation test is known to be relatively inefficient for the serodiagnosis of RS virus and *M. pneumoniae* infections of early life. The serological findings from tropical or semitropical areas confirm the general impression that RS virus is the most important respiratory tract pathogen of early life. Thus the pattern of infection with this agent resembled that seen in a number of urban populations in temperate areas. The importance of the parainfluenza viruses in the semitropical and tropical areas is of interest and again this pattern resembled that seen in urban temperate regions. The lesser importance of *M. pneumoniae* as a pathogen of early life is consistent with the pattern described for temperate areas. Influenza and adenoviruses were of moderate importance, while *C. burneti* and psittacosis-ornithosis agents were relatively unimportant as causes of severe lower respiratory disease in early life. The findings indicate that the pattern of severe respiratory disease is similar to that which has been defined for a number of large populations in temperate areas. Thus the effort to prevent respiratory disease in early life in temperate regions has relevance to the prevention of similar illnesses in tropical and semitropical areas where the mortality rate for children hospitalized with acute disease of the lower respiratory tract often approaches 50%. The importance of this problem is especially evident in certain tropical areas.

RÉSUMÉ

L'OMS, en étroite collaboration avec le Centre international OMS de Référence pour les maladies à virus des voies respiratoires autres que la grippe, à Bethesda, Etats-Unis d'Amérique, a organisé une étude dont le but était de définir l'étiologie virale des infections respiratoires graves chez les enfants, particulièrement dans les régions tropicales. L'étude a été divisée en deux phases. La première, au cours de laquelle seules des méthodes sérologiques ont été utilisées, a duré deux ans. Elle est maintenant terminée et ses résultats sont rapportés dans le présent article.

Onze laboratoires ont participé à cette première phase; ils sont situés dans les villes suivantes: Beyrouth, Bombay, Dakar, Hong Kong, Kingston, Le Caire, Lima, New Delhi, Porto, Port of Spain et Singapour. Des sérums couplés prélevés chez 528 enfants de moins de cinq ans, hospitalisés pour affections respiratoires graves dont on suspectait l'étiologie virale, ont été rassemblés et envoyés congelés au Centre de Bethesda. Dans ce Centre, des épreuves standard de fixation du complément ont été effectuées sur tous les sérums, en faisant appel aux antigènes suivants: virus paragrippal (types 1, 2 et 3), virus grippal (types A et B), adénovirus, virus respiratoire syncytial (VRS), *Mycoplasma pneumoniae*, *Coxiella burneti* et virus de la psittacose-ornithose. Des fiches contenant des informations cliniques accompagnaient les sérums.

Sur l'ensemble des enfants soumis à l'enquête, 41% présentaient une élévation du taux d'anticorps pour un ou plusieurs des agents suivants: VRS (19%), virus paragrippal (16%), dont 11% du type 3, 6% du type 1 et 2% du type 2, adénovirus (6%), virus grippal A (4%) et B (2%), *M. pneumoniae* (5%) et *C. burneti* et psittacose chacun moins de 1%. Dans 8% des cas, on observa une infection multiple. Dans la plupart des pays, une distribution semblable fut constatée. Le virus respiratoire syncytial prédominait chez les enfants en bas âge, particulièrement chez les enfants de moins d'un an et dans les cas de bronchiolite et de pneumonie. Le second rang était occupé par les virus paragrippaux qui furent souvent associés avec des cas de croup — mais ne furent pas décelés avec une fréquence particulière dans les groupes d'âges inférieurs. Les virus grippaux, les adénovirus et *M. pneumoniae* montrèrent une importance moyenne; le rôle de *C. burneti* et des agents de la psittacose-ornithose était relativement mineur. Ce genre de distribution ressemble beaucoup à celui que l'on observe habituellement dans les climats tempérés.

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